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Mating behaviour of the groundwater copepod *Parastenocaris phyllura* Kiefer, 1938 (Copepoda: Harpacticoida)

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Abstract

In the course of a cohort study of *Parastenocaris phyllura* several matings were observed under a microscope and recorded on video. In *Parastenocaris phyllura* no "precopulatory mate guarding" was found. Each mating behaviour comprises three phases and lasts for about 15 minutes, during which time the male actively moves its antennulae and antennae, pereiopods 1, 2, 3, 4, and furcal rami. The female is held in a characteristic position by the male and is entirely passive during the process.

Zusammenfassung

Innerhalb einer Kohortenstudie mit *Parastenocaris phyllura* wurde mehrmals das komplexe Paarungsverhalten mit Videotechnik und Mikroskop analysiert. Dadurch konnten die Bewegungen der einzelnen Extremitäten erfaßt, registriert und untersucht werden. Bei *Parastenocaris phyllura* wurde kein "precopulatory mate guarding" beobachtet. Jede Paarung besteht aus drei Phasen und dauert etwa 15 Minuten. Während dieses Vorganges ist das Männchen mit seinen Antennulae und Antennae, mit den Peraeopoden 1, 2, 3, 4 und Furkalästen aktiv. Das Weibchen wird vom Männchen in einer charakteristischen Stellung gehalten und ist während der Paarungsphasen völlig passiv.

Introduction

Harpacticoid copepods have long been recognized as important members of the meiofauna. Yet, not much is known of groundwater harpacticoid biology, especially about mating behaviour.

Mating behaviour in Calanoida, Cyclopoida, Harpacticoida, Poecilostomatoida and Siphonostomatoida has been overviewed (Lang, 1948; Hicks & Coull, 1983; Ridley, 1983; Boxshall, 1990). Schnitter & Chappuis (1915) made a few observations of mating behaviour in their first description of *Parastenocaris fontinalis*. Their results differ from observations on *Parastenocaris phyllura* Kiefer, 1938 (cf. Glatzel, 1990). These casual observations allow only a very general description of the process but are inadequate for detailed analysis of the function of the various appendages.

The mating process of *Parastenocaris phyllura* is very seldom observed and it has never been analysed in any species of Parastenocarididae or other groundwater harpacticoids. During mating male and female clasp together. Some of the appendages are moved rapidly and while the pereiopods of the male are rubbing, the pair can drift through the water. So, their position can vary and it is very difficult to analyse the intricate movements.

Parastenocaridids are noteworthy for their pronounced sexual dimorphism. In all appendages except antennae, mouthparts and pereiopods 2, but including furcal rami, the male differs from the female. It is obvious that these morphological differences of the male play a role in mating behaviour. The pereiopods 3 of the male have been named by several authors a "Kopulationsorgan", "Zange", "Greifzange", "Kopulationsfuß", or "pincer-like structure" (Kessler, 1914; Schnitter & Chappuis, 1915; Hertzog, 1936; Kiefer, 1938; Chappuis, 1940; Pesce et al., 1988). It has been speculated that the terminal "elements" of pereiopods 3 are used as a claw or tongs for spermatophore transfer or attachment to the female's gonopore. The pereiopods 3 are modified appendages but, so far,

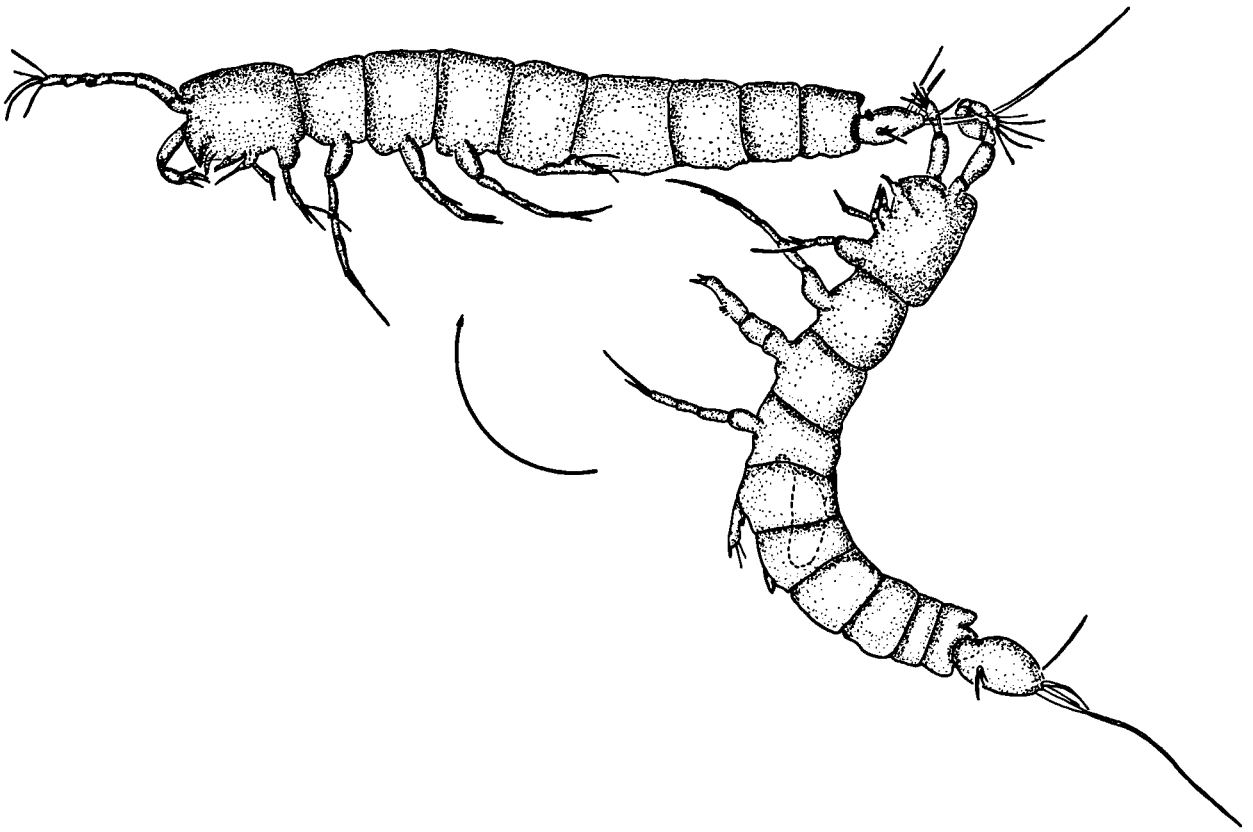

Fig. 1. Parastenocaris phyllura, the male (length: 470 µm) clasps the female's furcal setae with its antennula and swings to the female's ventral side (only left appendages are drawn).

their function in mating behaviour has not been correctly understood.

Material and methods

The specimens belong to the authors' laboratory culture which has its origin in sandy beaches of a lake named Großer Plöner See. Pairs of males and females of *Parastenocaris phyllura* have been cultured in a refrigerator in small glass petri dishes (3 × 1 cm) at 13°C.

For video analysis, the mating process was filmed with a "Hitachi" high resolution CCD camera fitted to a "Wild" macroscope M 420 (magnification: 80×) in a petri dish at a constant temperature or to a "Leitz Dialux 22" microscope (magnification: 125–200×) in a polished slide with a drop of water (6 mm in diameter). A professional "Panasonic" S-VHS time lapse video cassette recorder was used in combination with a "Sony" high resolution monitor for recording and for frame by frame analysis. The photographs (Figs. 2, 4, 5) are taken off the monitor.

Results

In *Parastenocaris phyllura* there is no precopulatory mate guarding: the adult male clasps only the adult female. The male and female lie in opposite directions with their ventral sides opposed. The male's cephalothorax lies opposite the female's anal somite and preceding somite (see Figs. 2, 3, 4).

There are three phases in mating behaviour:

1. *Initial phase*

The male uses its right geniculate antennula to grasp one of the female's furcal setae. Its left geniculate antennula searches for the other furcal seta of the female. The antennula grasps the seta from the inner side outwards (Fig. 1). When the male

grasps the seta of only one furcal ramus, both animals begin a series of loops, turns and somersaults and the female tries to remove the clasp with her mouthparts. Only rarely the female is able to escape. This phase lasts about two minutes.

2. *Stimulation/courtship phase*

After the male has grasped setae of both furcal rami it immediately rolls its abdomen dorsad, presses it to the female's ventral side and grasps the female exactly at the anterior end of her genital double-somite with both third pereiopods (Figs. 3, 4). The first pereiopods are held outstretched, lateral to the female's anal somite and the preceding somite. Simultaneously the male changes its grasp from antennulae to antennae. The antennulae are held ventrally, touching her anal somite and the preceding somite. Before the male clasps the female with its furca, the male searches with it ventrally over the female's pereiopods from anterior to posterior and stops exactly between pereiopods 3 and 4.

After placing the female into the courtship position, the male's antennae begin to paddle each furcal ramus of the female (approximately 900 paddles per minute). The antenna grasps the furcal ramus between the strong inner terminal seta and the posterolateral seta. Shortly afterwards the second pereiopods of the male start to rub rapidly back and forth against the lateral surface of the female's genital double-somite and the two following ones (approximately 540 rubs per minute).

The fourth pereiopods of the male beat on the female's sixth thoracic somite ventrally with double strokes, forward and backward, on the female's sixth thoracic somite (approximately 60 strokes per minute). While the pereiopods of the male are making these stroking movements the pair can drift through the water. This second phase lasts about nine minutes and is summarized in Table I.

3. *Copula phase*

When the spermatophore is extruded, bottom first, from the male's gonopore at the end of the genital somite (the 7th body somite) (Fig. 5), the male ceases its movements and relaxes its grasp of the furcal rami for a moment. At first the bottom of the spermatophore is directed to the male's furcal rami and turns by itself with the bottom caudad to the female's furcal rami. The spermatophore is placed between the male's and female's ventral surface. It is attached exactly at the female's gonopore (cf.

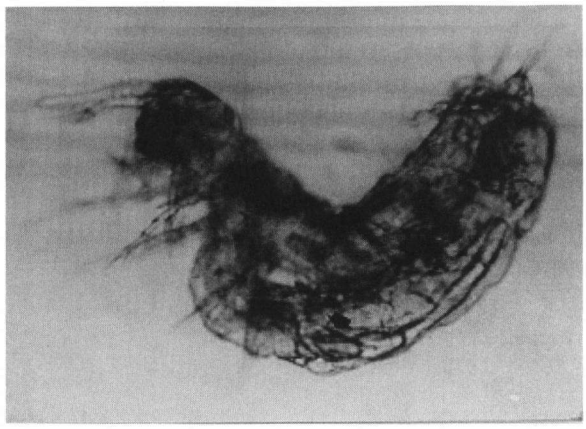

Fig. 2. Parastenocaris phyllura, courtship, male below, with its spermatophore (arrow) inside, female above (125 ×).

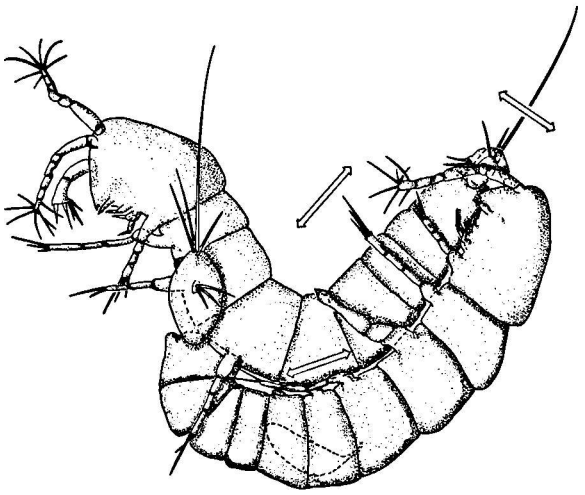

Fig. 3. Parastenocaris phyllura, courtship. This is an association after the male clasps the female and before copula, the stimulation/courtship phase. The female lies on top, the male below. Some of the appendages are moving rapidly. The arrows indicate the moving direction of the male's appendages activity (antennae 900 paddles/minute, second pereiopods 540 rubs/minute, fourth pereiopods 60 knocks/minute).

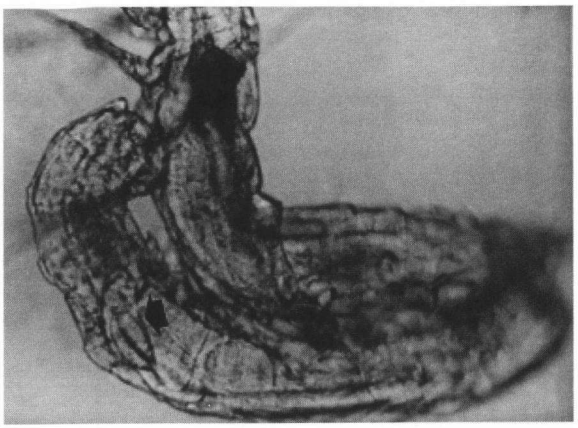

Fig. 4. Parastenocaris phyllura, courtship, male below with spermatophore (arrow), female above (200 ×).

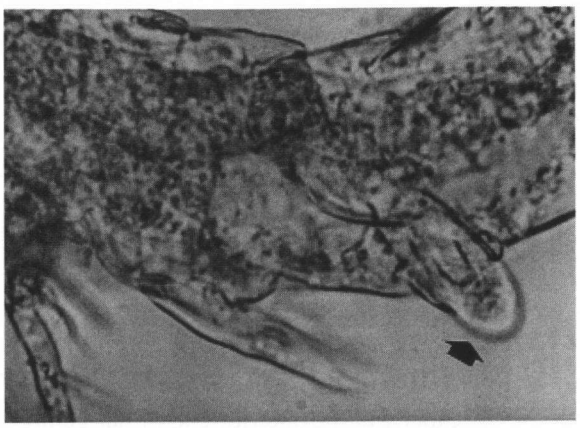

Fig. 5. Parastenocaris phyllura, male, lateral view, extrusion of the spermatophore (arrow), coming out of the male's gonopore with its bottom first at the end of the genital somite (500 ×).

Table I. Stimulation/courtship phase of female and male *Parastenocaris phyllura*. The female and male lie in opposite directions with their ventral sides opposed. The male's cephalothorax lies opposite the female's anal somite and preceding somite (Figs. 2, 3).

	male	female
antennulae	ventrally touching female's anal and preceding somite	outstretched
antennae	paddle the furcal rami of female	–
pereiopods 1	laterally between female's anal- and preceding somite	outstretched
pereiopods 2	rub against lateral surface of female's genital double-somite and two following somites	outstretched
pereiopods 3	grasp female's body dorsally and laterally at anterior end of genital double-somite	frontally outstretched
pereiopods 4	knock ventrally with double strokes	held outstretched lateral to male's abdominal somite 2
pereiopods 5	–	–
furca	grasps female between her pereiopods 3 & 4	–

Glatzel, 1990) by means of an adhesive material. When the spermatophore comes out, the male presses it with its body towards the female's ventral surface and grasps her again with its furca between the pereiopods 3 and 4. The time taken for discharge of the spermatophore is unknown.

In most cases clasping of the female is followed by a courtship and copula and the mating sequence ends with a three minute re-clasping (see Dürbaum, 1995). Then, about three minutes after copula, the female flexes her cephalothorax ventrally and dorsally, and strokes with pereiopods 2 and 3 removing the male's clasp.

A new spermatophore is produced within 12–24 hours (at culture temperature) and the distinct two-phases contents of the spermatophore are visible through the light microscope after 24 hours. Remating has been observed several times in cultures. The first spermatophore is produced approximately seven days or more after the terminal moult of the male.

Discussion

In *Parastenocaris phyllura* the male strongly differs from the female in antennulae, pereiopods 3 and 4, and furcal rami. Boxshall (1990) pointed out that the antennulae, which are used during mating, are in general strongly sexually dimorphic in male harpacticoids. We also found that the antennulae are important during the initial phase when the male grasps the female (see Haq, 1972; Kern et al., 1984). In Parastenocaridids the male's third pereiopods are typically strongly sexually dimorphic and are important in the mating behaviour of

Parastenocaris phyllura when the male clasps the female with both third pereiopods to press her to his body. Also the fourth pereiopods play a role in the stimulation phase, but we could not identify the function of the comb-like endopods of the fourth pereiopods, which are reduced or lost in some species of this family. The antennae of female and male are not sexually dimorphic. They are used for locomotion (Glatzel, 1990) and play an important part in clasping the female and paddling the furcal rami during the stimulation phase. It is difficult to determine whether the male "stimulates" himself or the female or both.

The male has three fixing points to clasp the female during courtship: (1) gripping her furcal rami with the antennae, (2) pressing the female to his body with his third pereiopods, and (3) clasping the female's body with his laterally compressed furcal rami. It is assumed that there is a fourth fixing point using the ventrofrontal process of the male's fourth pereiopods (see Glatzel, 1991), located frontally between the female's fifth pereiopods.

In *Parastenocaris phyllura* the male uses his antennulae only to clasp the female's furcal setae in the initial phase, but not during courtship and copula (see Huys & Boxshall, 1991: 331). The grip of the antennulae changes while clasping the antennae. These examples show that strongly dimorphic appendages are indicative of a function in mating behaviour, but only observation of the total mating process can reveal whether the inference is correct or not.

We observed a re-clasping for about 3 minutes after copula was finished. The male claps the female again in the courtship position without moving its appendages. Dürbaum (1995) called this behaviour "postcopulatory mate guarding – phase". The male guards the female at least until the spermatophore is discharged, but there was one exception in his study, viz. *Tisbe battagliai* Volkmann-Rocco, 1972.

We could not determine the time for discharge of the spermatophore in *Parastenocaris phyllura* during re-clasping, but in our opinion this behaviour phase serves to ensure that the spermatophore is not dislodged before it discharges into the female's seminal receptacles (to secure paternity).

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